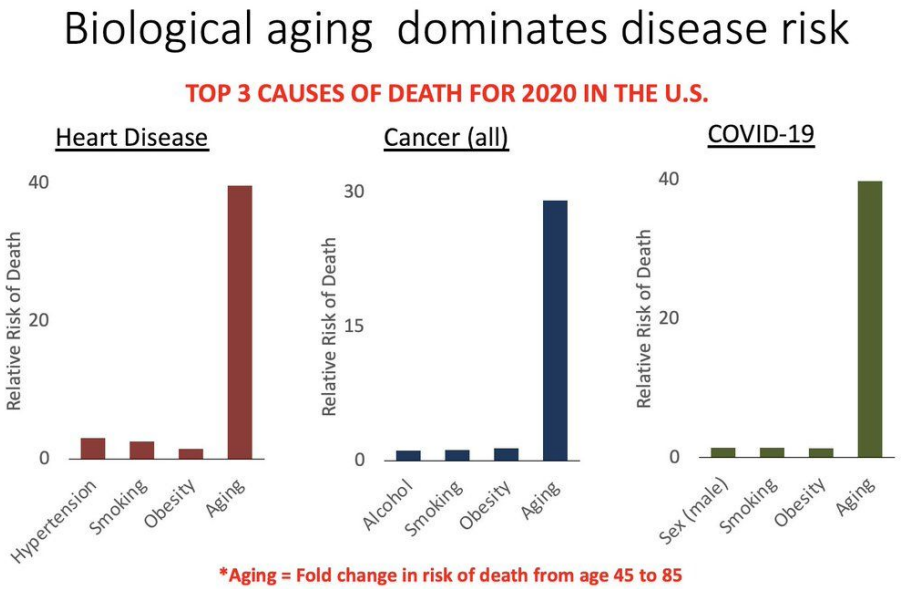
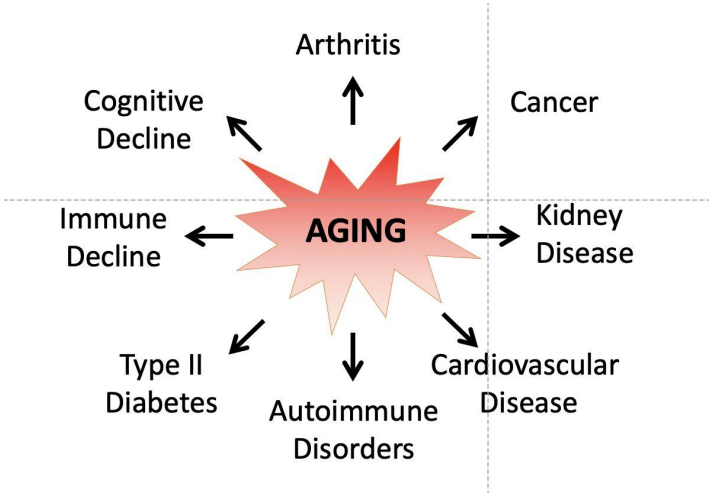
TruDiagnostic™
The Epigenetic Company

There are many ways to measure aging: **What test/algorithm is best and why?**

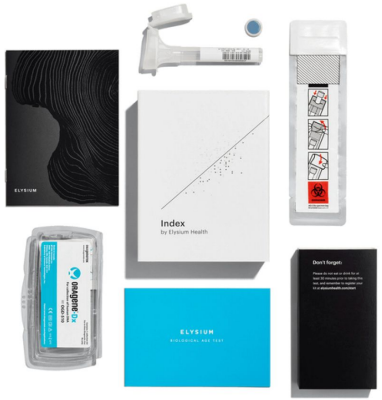
We All Know Why Measuring the Biological Aging Process Matters: Aging Sucks

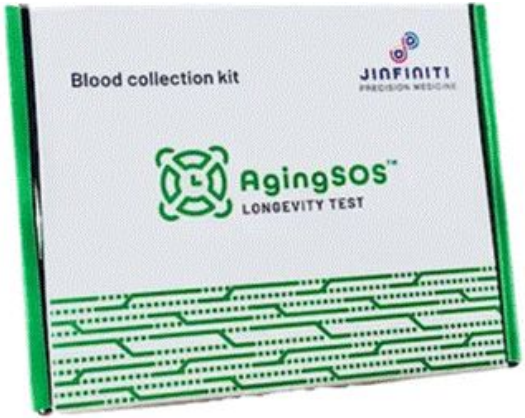




What Commercial Biological age tests are available?

*Lab visit required



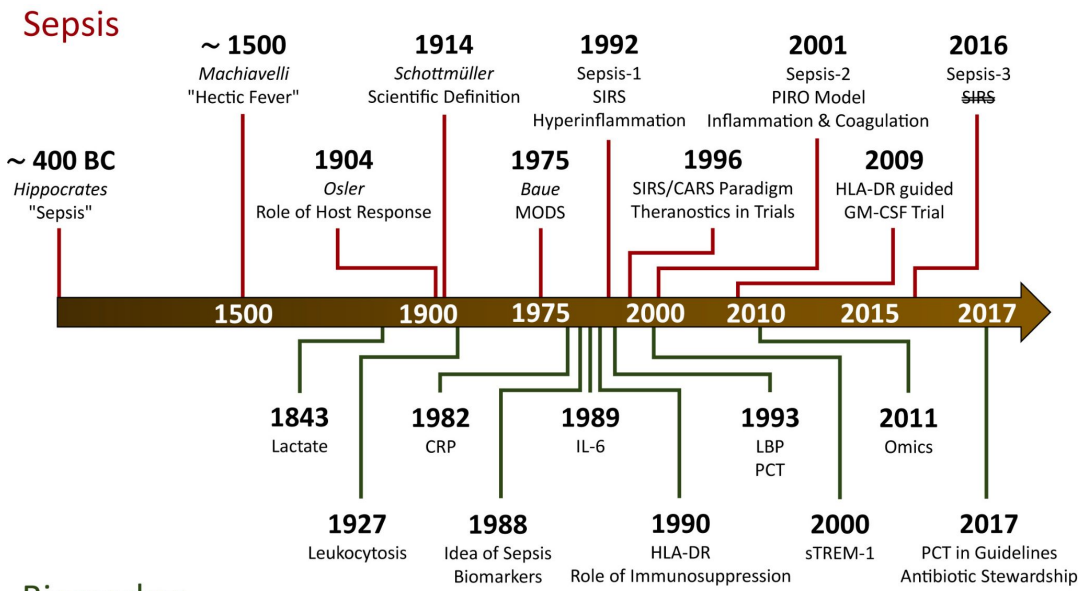


InsideTracker

Repeat DX

TELOYEARS™

What is a Biomarker?



Biomarkers have been defined as: “indicators of biological and pathogenic processes, or pharmacologic responses to a therapeutic intervention that defines what is normal while predicting or detecting what is abnormal.”

The History Of Biological Age Measurements

During the past decades, extensive effort has been made to identify such aging biomarkers that, according to the stage-setting definition (Baker and Sprott, 1988), are “biological parameters of an organism that either alone or in some multivariate composite will, in the absence of disease, better predict functional capability at some late age, than will chronological age”. Later on, the American Federation for Aging Research (AFAR) formulated the criteria for aging biomarkers as follows:

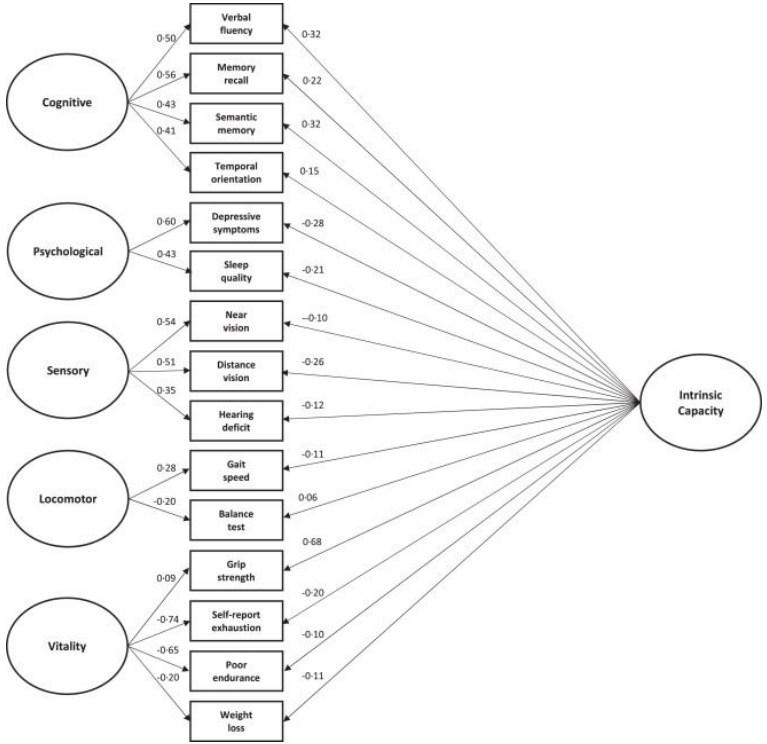
1. It must predict the rate of aging. In other words, it would tell exactly where a person is in their total life span. It must be a better predictor of life span than chronological age.

2. It must monitor a basic process that underlies the aging process, not the effects of disease.

3. It must be able to be tested repeatedly without harming the person. For example, a blood test or an imaging technique.

4. It must be something that works in humans and in laboratory animals, such as mice. This is so that it can be tested in lab animals before being validated in humans.

What is Biological Aging?
How do we best measure it?

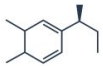


Musculoskeletal changes
- ⬆ Adiposity
- ⬇ Muscle mass
- ⬇ Grip strength
- ⬇ Bone-mineral density
- ⬇ Gait velocity
- ⬇ Body weight

Stem-cell changes
- ⬇ % COP
- ⬇ COP lamin A

Serum markers
- ⬇ Hemoglobin
- ⬆ Albumin
- ⬆ Oxidation products
- ⬇ Antioxidants

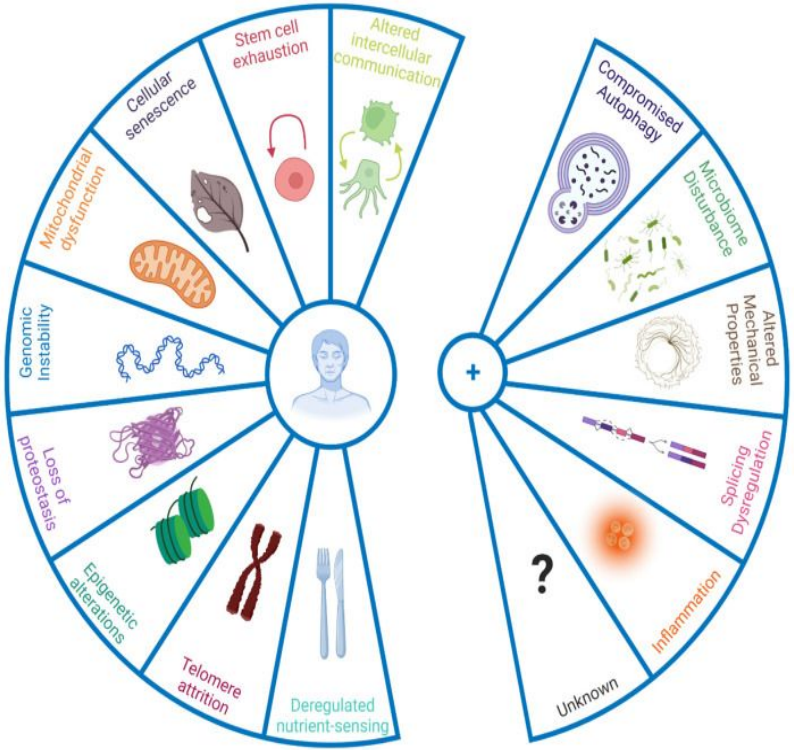
Metabolic markers
- ⬆ HbA_{1c}



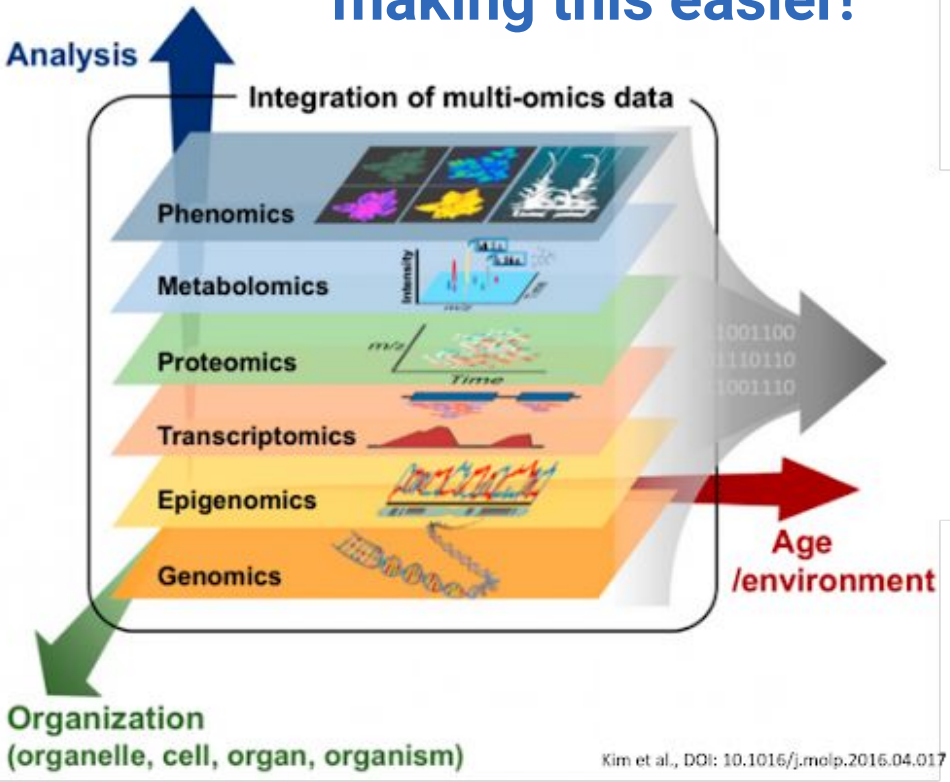
Hormonal changes
- ⬇ DHEA
- ⬇ Testosterone
- ⬇ Vitamin D
- ⬆ PTH
- ⬇ IGF1

Inflammatory markers
- ⬆ CRP
- ⬆ IL6
- ⬆ TNFα

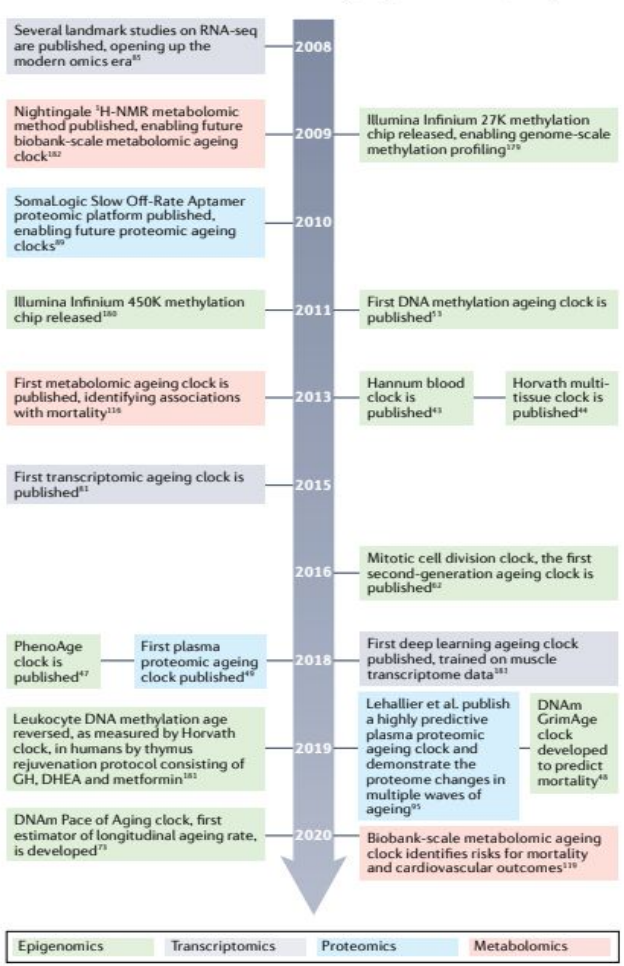
Aging is EXTREMELY Complex



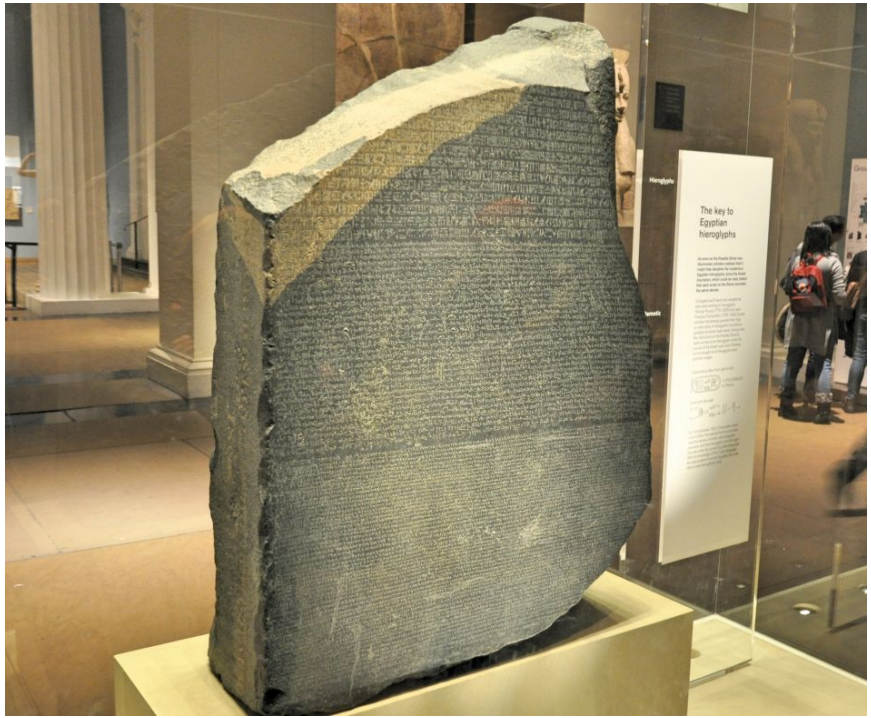
The Advent of AI and Computer Learning is making this easier!

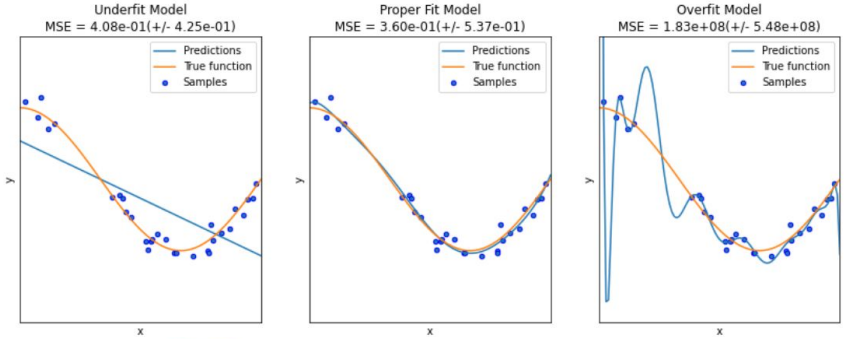


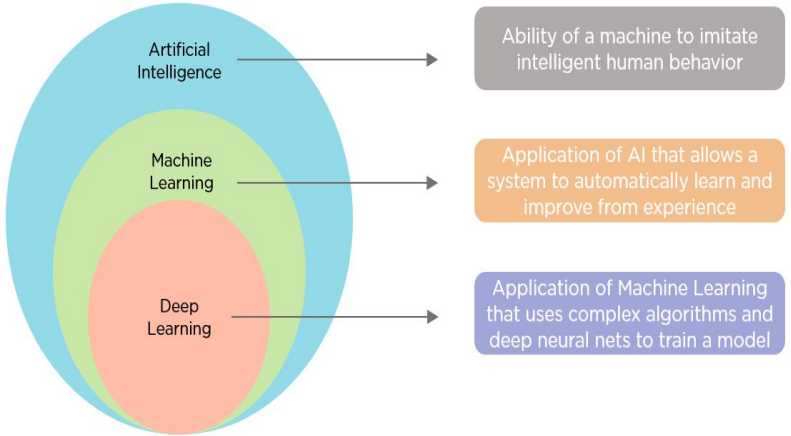
Kim et al., DOI: 10.1016/j.molp.2016.04.017



Measurement means nothing without interpretation of the data







Proteomics Clocks

"There were cases of substantial divergence between participants' chronological and physiological age — for example, among the subjects in the LonGenity study, with their genetic proclivity toward exceptionally good health in what for most of us is advanced old age.

"We had data on hand-grip strength and cognitive function for that group of people," Wyss-Coray. "Those with stronger hand grips and better measured cognition were estimated by our plasma-protein clock to be younger than they actually were."

<u>However, the protein-derived age variable itself was not tested for associations with health outcomes.</u>

Stanford scientists reliably predict people's age by measuring proteins in blood

Protein levels in people's blood can predict their age, a Stanford study has found. The study also found that aging isn't a smoothly continuous process.

DEC 5
2019

The carnival worker who tries to guess your age relies on aspects of your appearance, such as your posture and whether any wrinkles emanate from the corners of your eyes and lips. If the carny's guess is more than a few years off, you win a stuffed koala.

But a team of Stanford University School of Medicine scientists doesn't need to know how you look to guess your age. Instead, it watches a kind of physiological clock: the levels of 373 proteins circulating in your blood. If the clock is off, you don't win a plush toy. But you may find out important things about your health.

Tony Wyss-Coray is the senior author of a study that found protein levels in people's blood can predict their age.
Norbert von der Groeben

"We've known for a long time that measuring certain proteins in the blood can give you information about a person's health status — lipoproteins for cardiovascular health, for example," said Tony Wyss-Coray, PhD, professor of neurology and neurological sciences, the D. H. Chen Professor II and co-director of the Stanford Alzheimer's Disease Research Center. "But it hasn't been appreciated that so many different proteins' levels — roughly a third of all the ones we looked at — change markedly with advancing age."

Methylation as a Biomarker Beyond Aging

> 5 to 10 years from now, the health system that doesn't use this data to improve their medical delivery is going to be deemed archaic.
>
> **- Atul Butte,**
> Biomedical Informatics
> Researcher in Silicon Valley

Arthritis & Rheumatology
AN OFFICIAL JOURNAL OF THE AMERICAN COLLEGE OF RHEUMATOLOGY
American College of Rheumatology

Full Length

Peripheral blood DNA methylation-based machine learning models for prediction of knee osteoarthritis progression: biospecimens and data from the Osteoarthritis Initiative and Johnston County Osteoarthritis Project

Christopher M. Dunn MS, Cassandra Sturdy BS, Cassandra Velasco BS, Leoni Schlupp BS, Emmaline Prinz BS, Vladislav Izda BS, Liubov Arbeeva MS ... See all authors

First published: 12 August 2022 | https://doi.org/10.1002/art.42316

CLINICAL AND TRANSLATIONAL MEDICINE
Open Access

RESEARCH ARTICLE | Open Access

Comprehensive methylome sequencing reveals prognostic epigenetic biomarkers for prostate cancer mortality

Ruth Pidsley, Dilys Lam, Wenjia Qu, Timothy J. Peters, Phuc-Loi Luu, Darren Korbie, Clare Stirzaker, Roger J. Daly, Phillip Stricker, James G. Kench, Lisa G. Horvath, Susan J. Clark

First published: 30 September 2022 | https://doi.org/10.1002/ctm2.1030

Methylation risk scores are associated with a collection of phenotypes within electronic health record systems

Mike Thompson, Brian L. Hill, Nadav Rakocz, Jeffrey N. Chiang, IPH, Sriram Sankararaman, Ira Hofer, Maxime Cannesson, Noah Zaitlen, Eran Halperin

doi: https://doi.org/10.1101/2022.02.07.22270047

Now published in *npj Genomic Medicine* doi: 10.1038/s41525-022-00320-1

Proteomics as a Biomarker

SAMPLE REPORT

somalogic

Elliot Everson
Accession Number: A00005

Your Test Results Summary (continued)

Body Fat Percentage
What is my body fat percentage?

29%
LESS | AVERAGE | MORE

Visceral Fat
How much fat is around my organs?

5.49 lbs
LOW | AVERAGE | HIGH

Lean Body Mass
What is my lean body mass?

140.5 lbs
MORE | AVERAGE | LESS

Heart Failure Prognosis - HFrEF - 6 months
What is my heart failure prognosis in the next 6 months?

5%
Low | Medium | High

Heart Failure Prognosis - HFrEF - 12 months
What is my heart failure prognosis in the next 12 months?

5%
Low | Medium | High

Heart Failure Prognosis - HFpEF - 6 months
What is my heart failure prognosis in the next 6 months?

3%
Low | Medium | High

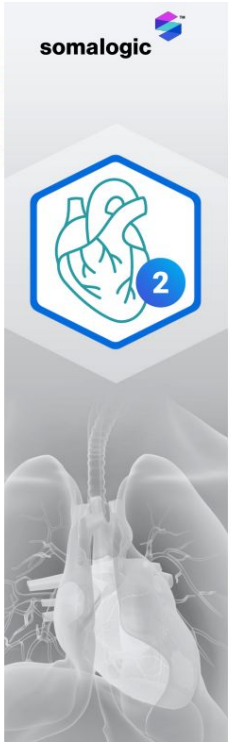
Heart Failure Prognosis - HFpEF - 12 months
What is my heart failure prognosis in the next 12 months?

5%
Low | Medium | High

SAMPLE REPORT

somalogic

Elliot Everson
Accession Number: A00005



Secondary Cardiovascular Risk — 4 years

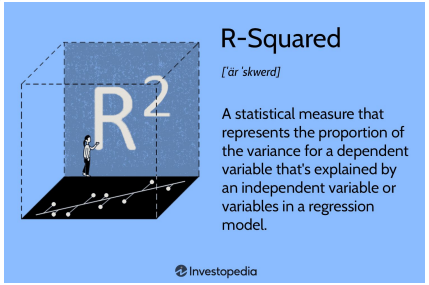
What is my risk of having a heart attack, stroke, or heart failure within the next 4 years?

You may have already had a heart attack or have specific risk factors if your healthcare provider has ordered this test for you. This test helps define your risk for a problem with your heart or having a stroke in the future. Understanding your results can help you and your healthcare provider address the factors that you can change to reduce that risk.

15%
LOW | **MEDIUM** | HIGH

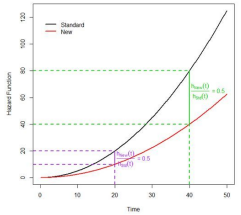
You have a MEDIUM risk of having an issue with your heart or a stroke in the next 4 years. In our test population, 15 in 100 people with a similar result to yours had an event within 4 years.

What are our tools to validate which of these Omics is best?



Hazard Ratios

- Hazard ratio = $\frac{\text{hazard function New}}{\text{hazard function Std}}$
- Makes assumption that this ratio is constant over time



ICC	Agreement
1.0	Perfect agreement
0.99 to 0.81	Almost perfect agreement
0.80 to 0.61	Substantial agreement
0.60 to 0.41	Moderate agreement
0.40 to 0.21	Fair agreement
0.20 to 0.01	Slight agreement
0.0 to –0.1	Poor agreement

How widely has this been validated?

Phenotypically trained?

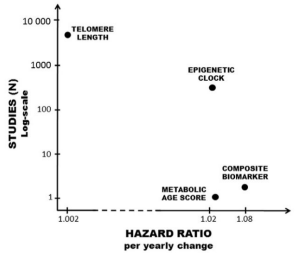


Fig. 2. Number of studies versus mortality hazards for the biological age predictors. Overview of the four biological age predictors telomere length (Rode et al., 2015), epigenetic clock (Chen et al., 2016), Metabolic Age Score (Hertel et al., 2016), and composite biomarker (Levine, 2013) which have all been used in survival models. The hazard ratio per yearly change in biological age (de-)acceleration for each predictor is presented on the x-axis. The y-axis presents an approximation of the number of studies on a log-scale where the predictor has been used.

Do these respond to interventions we know beneficially affect biology of aging? (Separate of disease)

Biomarker Criteria	Horvath epigenetic age	Hannum epigenetic age	GrimAge	PhenoAge	DunedinPoAm
DNA Methylation Biomarker Calibrated to Detect:	Chronologic Age	Chronologic Age	Biomarkers, Smoking, Death	Phenotypic Age	Pace of Aging (change)
Feasible for use in a clinical trial in older adults?	✓	✓	✓	✓	✓
Robustly associated with chronological age across independent cohorts?	✓	✓	✓	✓	✓
Predict age-related change in function, chronic disease, or death?	✓	✓	✓	✓	✓
Responsive to interventions that beneficially affect the biology of aging?	--	--	--	--	--

There is already a Consensus of the Best Aging Clock…

Other “Omics” Age Predictors

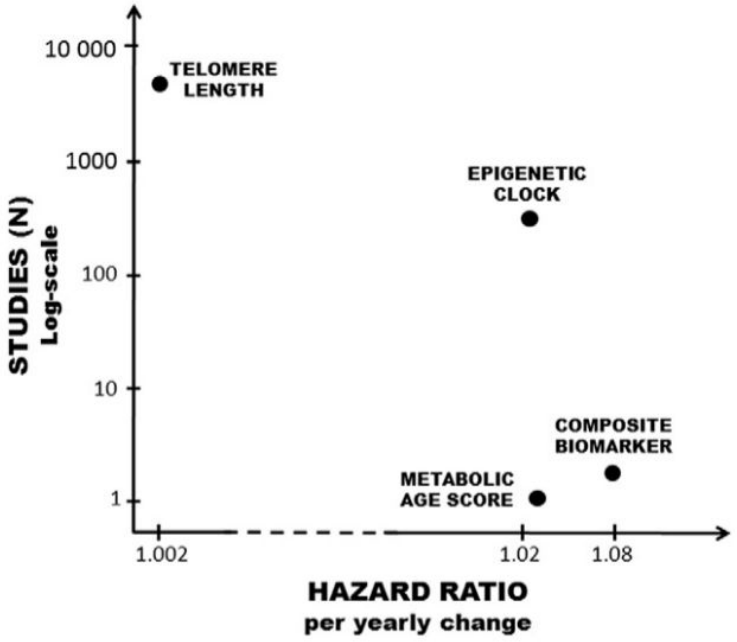
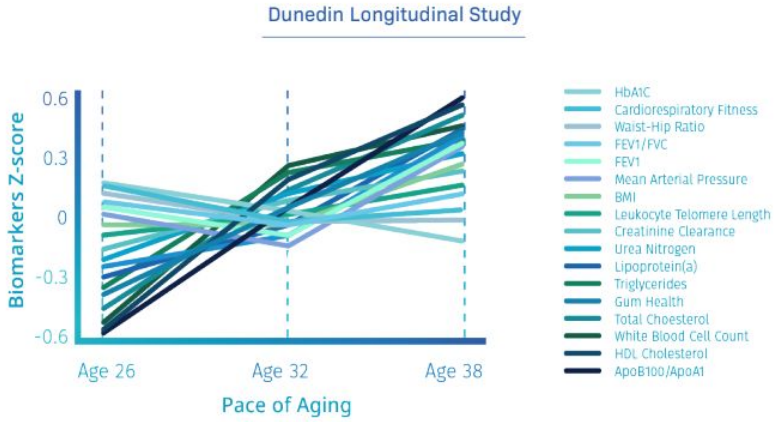


Fig. 2. Number of studies versus mortality hazards for the biological age predictors. Overview of the four biological age predictors telomere length (Rode et al., 2015), epigenetic clock (Chen et al., 2016), Metabolic Age Score (Hertel et al., 2016), and composite biomarker (Levine, 2013) which have all been used in survival models. The hazard ratio per yearly change in biological age (de-)acceleration for each predictor is presented on the x-axis. The y-axis presents an approximation of the number of studies

Table 1
Summary of biological age predictors.

Predictor	Method	Studies, N	Age-associated outcome	References
DNAmAge	DNA methylation	100+	Mortality, frailty, cognition, physical function, self-rated health, AD, PD, cancer	Horvath (2013), Hannum et al. (2013)
Telomere length	qPCR (T/S-ratio), Sothern blot (bp)	1000+	Mortality, cancer, CVD, AD, physical function, cognition	Blackburn et al. (2006)
Transcriptomic age	Gene expression	2	IL-6, urea, albumin, muscle strength, blood pressure, lipids, glucose, BMI, smoking	Holly et al. (2013), Peters et al. (2015)
Glycan age	Glycans, proteomics	1	Fibrinogen, HbA1c, BMI, triglycerides, uric acid	Kristic et al. (2014)
Protein-derived age	Proteomics	1	Low birth weight, Framingham risk score	Menni et al. (2015)
C-glyTrp	Metabolomics	1	Lung function, hip bone mineral density	Menni et al. (2013)
Metabolic age score	Metabolomics	1	Mortality, kidney function, HbA1c, hyperglyceridemia	Hertel et al. (2016)
Composite biomarker	10 biomarkers combined	3	Mortality, IQ, physical function	Levine (2013), Belsky et al. (2015)
Composite biomarker	19 biomarkers in a clustering approach	1	Mortality, cancer, CVD, T2D, physical function, cognition	Sebastiani et al. (2017)

AD, Alzheimer's Disease; PD, Parkinson's Disease; CVD, cardiovascular disease; T2D, type 2 diabetes; IL-6, interleukine 6; BMI, body mass index.



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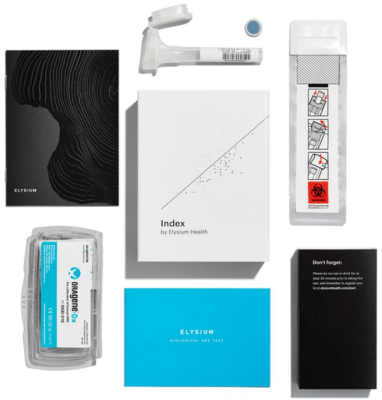
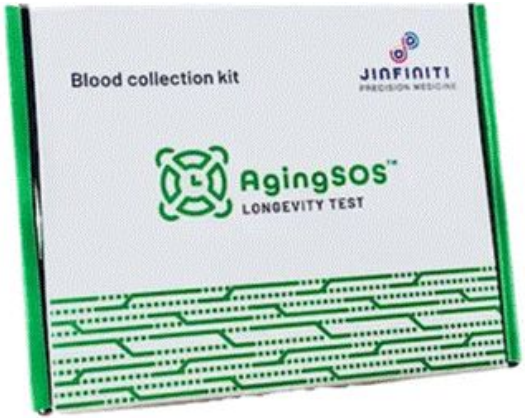




InsideTracker

Repeat DX

TELOYEARS™

Methylation: Strength and Weaknesses

Strengths	Weaknesses
• Highest ICC Values • Highest Hazard Ratios to Disease • Extremely Well Validated • Most Interventional Studies • Phenotypically traiined • Commercially Validated Algorithms Available • Many Different Reporting Insights	• 1st generation clocks might not respond to validated anti-aging interventions • Immune cells can confound • Causal? • Precision has been traditionally poor • Difference among the many clocks are confusing

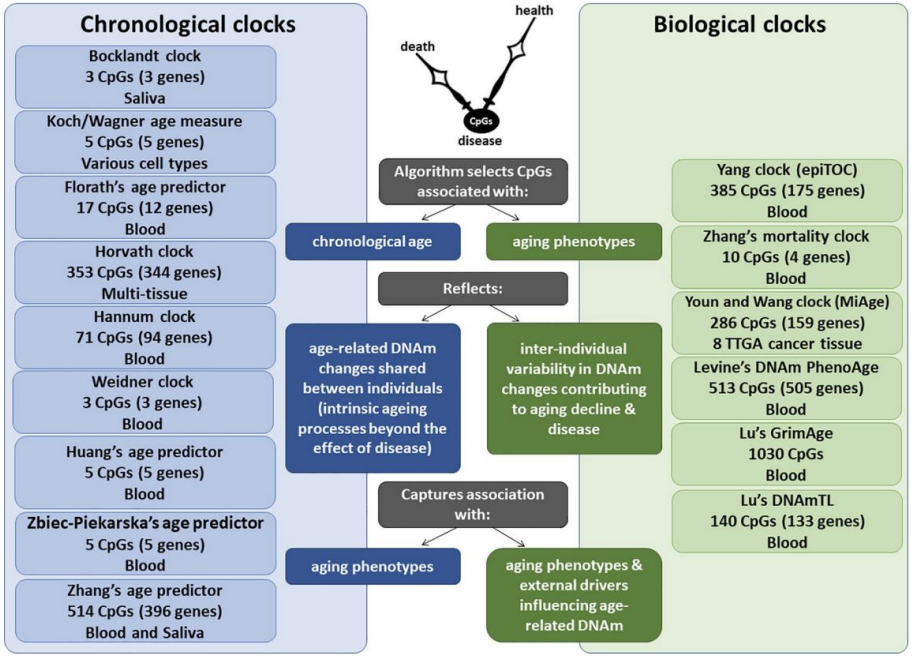
The History of Epigenetic Clocks

Landscape of Clocks

1st Gen. Clock
Trained using **Chronological Age**
Age ~ CpG Methylation + Age + Sex + …

2nd Gen. Clock
Trained using **Aging Phenotypes**
Biomarker ~ CpG Methylation + Age + Sex + …

3rd Gen. Clock
Trained using Aging Phenotype and measurements, **produces a instantaneous rate of aging**



Bergesma and Rogaeva, 2020

The DunedinPACE is the Most Predictive Clock

Time-to-Event Analysis of Mortality, Cardiovascular Disease (CVD) Diagnosis, and Stroke or Transient Ischemic Attack (TIA)

	Mortality			CVD			Stroke/TIA		
	HR	95% CI	p-value	HR	95% CI	p-value	HR	95% CI	p-value
DunedinPACE	1.64	[1.48-1.82]	6.46E-22	1.39	[1.25-1.54]	8.08E-10	1.42	[1.18-1.70]	1.57E-04
Horvath Clock	1.02	[0.95-1.10]	0.584	1.04	[0.95-1.14]	0.429	1.00	[0.86-1.17]	0.998
DunedinPACE	1.60	[1.43-1.78]	8.59E-17	1.34	[1.21-1.49]	1.73E-08	1.35	[1.13-1.61]	0.001
Hannum Clock	1.09	[1.01-1.17]	0.019	1.12	[1.02-1.23]	0.020	1.17	[1.00-1.36]	0.052
DunedinPACE	1.57	[1.40-1.75]	2.11E-15	1.35	[1.21-1.50]	9.25E-08	1.33	[1.07-1.65]	0.011
PhenoAge Clock	1.14	[1.03-1.25]	0.009	1.10	[1.00-1.20]	0.053	1.20	[1.02-1.40]	0.025
DunedinPACE	1.24	[1.12-1.38]	6.89E-05	1.18	[1.05-1.34]	0.007	1.33	[1.05-1.69]	0.019
GrimAge Clock	1.61	[1.49-1.74]	1.30E-33	1.33	[1.19-1.49]	5.74E-07	1.11	[0.91-1.35]	0.295

Repeated Measures Analysis of Incident Limitation to Activities of Daily Living (ADLs)

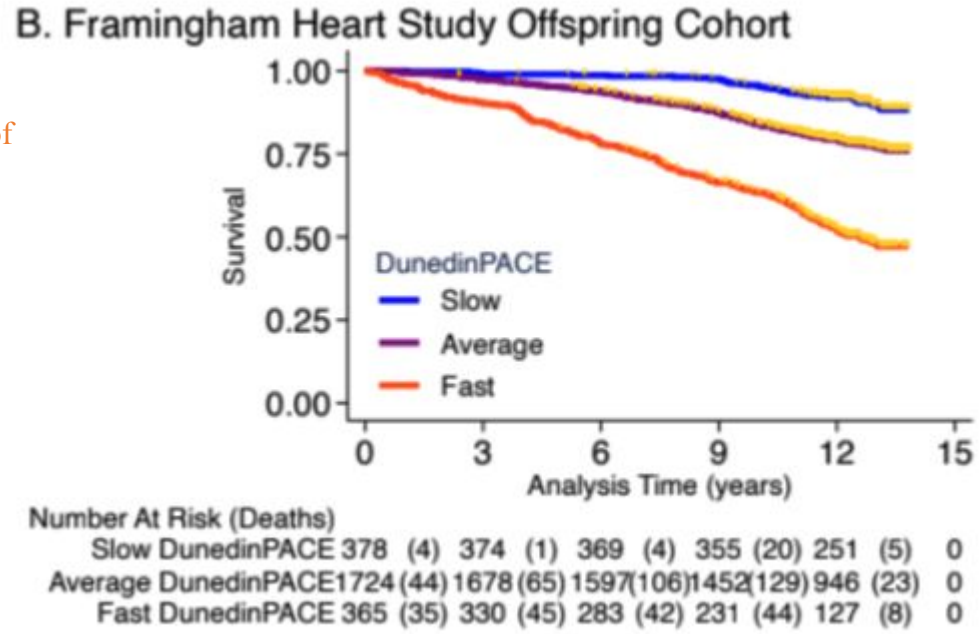
	Nagi ADLs			Katz ADLs			Rosow-Breslau ADLs		
	IRR	95% CI	p-value	IRR	95% CI	p-value	IRR	95% CI	p-value
DunedinPACE	1.39	[1.17-1.65]	1.49E-04	1.31	[1.14-1.50]	1.02E-04	1.40	[1.24-1.57]	2.36E-08
Horvath Clock	1.05	[0.88-1.26]	0.565	1.11	[0.98-1.26]	0.091	0.96	[0.89-1.05]	0.385
DunedinPACE	1.37	[1.14-1.64]	6.63E-04	1.30	[1.13-1.51]	3.07E-04	1.37	[1.20-1.57]	2.84E-06
Hannum Clock	1.08	[0.91-1.28]	0.381	1.06	[0.94-1.19]	0.367	1.04	[0.91-1.19]	0.562
DunedinPACE	1.40	[1.14-1.72]	0.001	1.26	[1.06-1.50]	0.007	1.43	[1.25-1.64]	3.54E-07
PhenoAge Clock	1.00	[0.77-1.30]	0.973	1.13	[0.95-1.35]	0.161	0.93	[0.81-1.07]	0.298
DunedinPACE	1.27	[1.02-1.58]	0.032	1.26	[1.02-1.54]	0.029	1.27	[1.08-1.50]	0.005
GrimAge Clock	1.18	[0.94-1.48]	0.158	1.10	[0.90-1.34]	0.357	1.15	[0.97-1.37]	0.098

DunedinPACE associations with health-span endpoints were little-changed by covariate adjustment for the Horvath, Hannum, or PhenoAge Clocks. In models adjusted for GrimAge, DunedinPACE associations with mortality, CVD, and disability were attenuated, but remained statistically different from zero (mortality HR = 1.24 [1.49–1.74], CVD HR = 1.18 [1.05–1.34], Nagi ADL IRR = 1.27 [1.02–1.58], Katz ADL IRR = 1.26 [1.02–1.54], Rosow-Breslau ADL IRR = 1.27 [1.08–1.50]); associations with stroke were similar to unadjusted models (HR = 1.33 [1.05–1.69]). Results for all models are reported in Supplementary file 1C. Thus, Dunedin PACE adds incremental prediction over and above all clocks studied here."

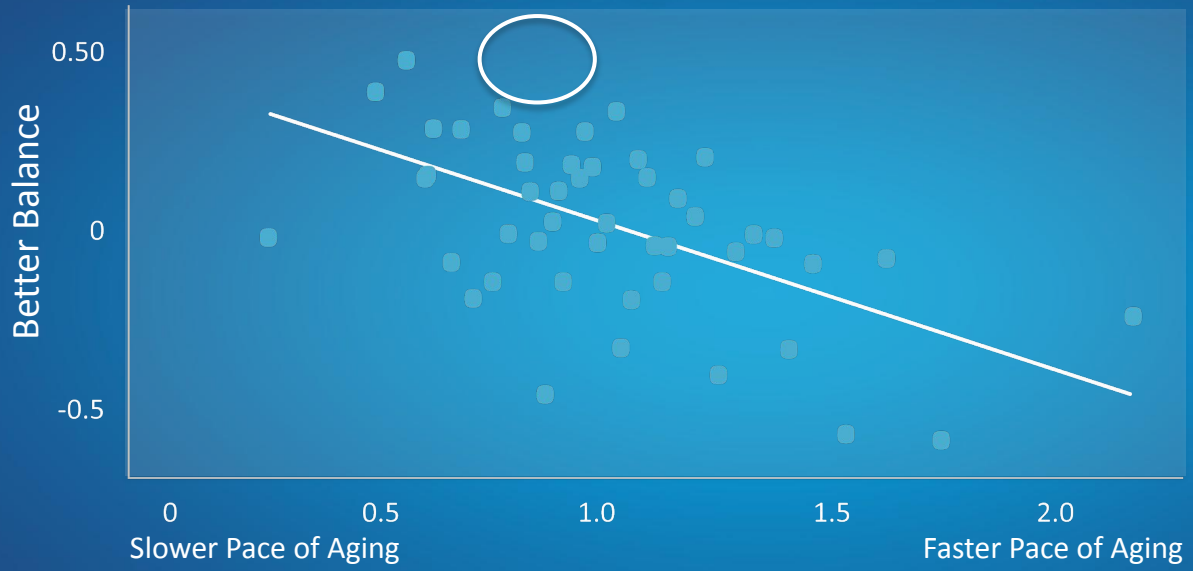
Nagi ADL Scale. Count of activities for which participants reported a lot of difficulty or inability to perform.
Pulling or pushing large objects
Stooping, crouching, or kneeling
Reaching or extending arms below shoulder level
Reaching or extending arms above shoulder level
Writing, handling, or fingering small objects
Standing in one place for long periods (15 minutes)
Sitting for long periods (one hour)
Lifting or carrying weights under 10 lbs
Lifting or carrying weights over 10 lbs
Katz ADL Scale. Count of activities for which participants required assistance or could not do themselves.
Dressing
Bathing
Eating
Transferring (getting in and out of a chair)
Toileting
Rosow-Breslau ADL Scale. Count of activities participants were not able to do.
Heavy work around the house
Walk half a mile without assistance
Walk up and down one flight of stairs

The DunedinPACE is the Most Predictive Clock

Dunedin Study members with methylation data at age 45, N = 817 had measured Pace of Aging (M = 0.99, SD = 0.30). This group formed the analysis sample to develop DunedinPACE.



One-leg Balance Test

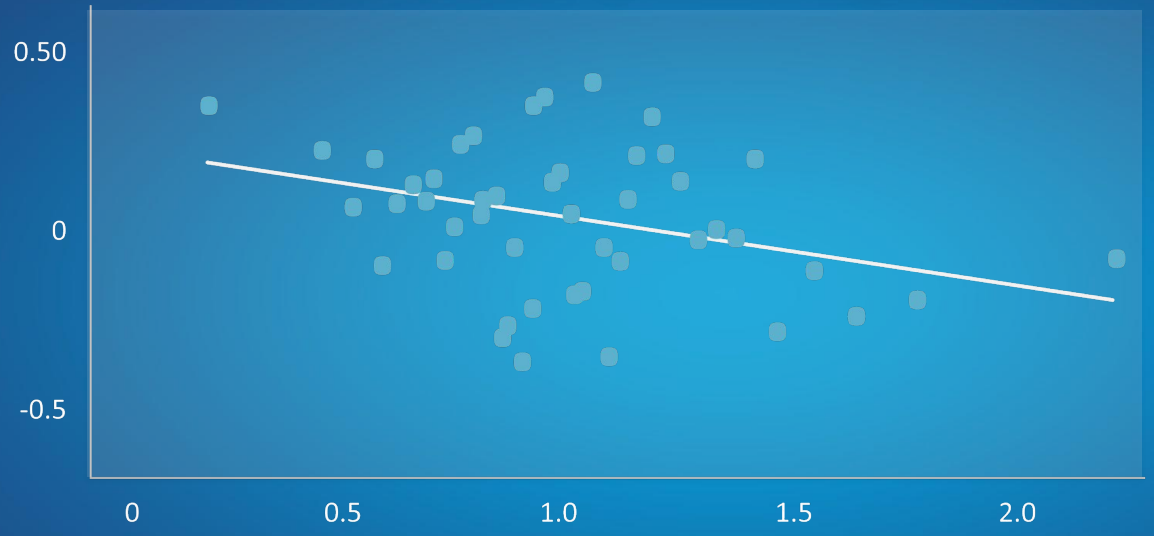


Pace of Aging 26 - 45 R = 0.36, $p < 0.001$

Each plotted point represents 20 study members

TruDiagnostic™

Grip Strength



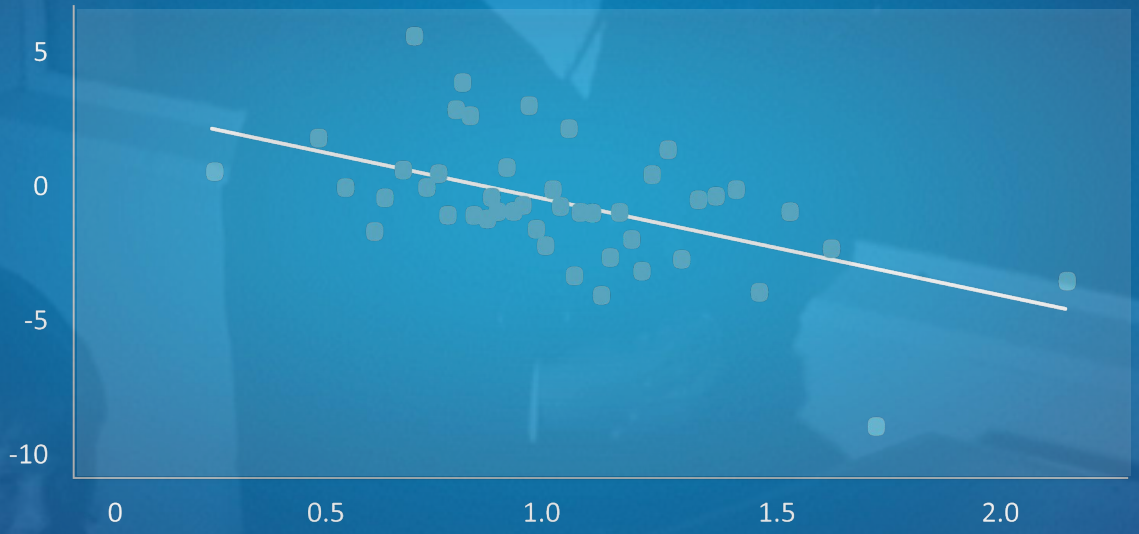
Pace of Aging 26 - 45 R = 0.07, p = 0.033

Each plotted point represents 20 study members

TruDiagnostic™

Cognitive Decline

(IQ Change from Childhood to Age 45)

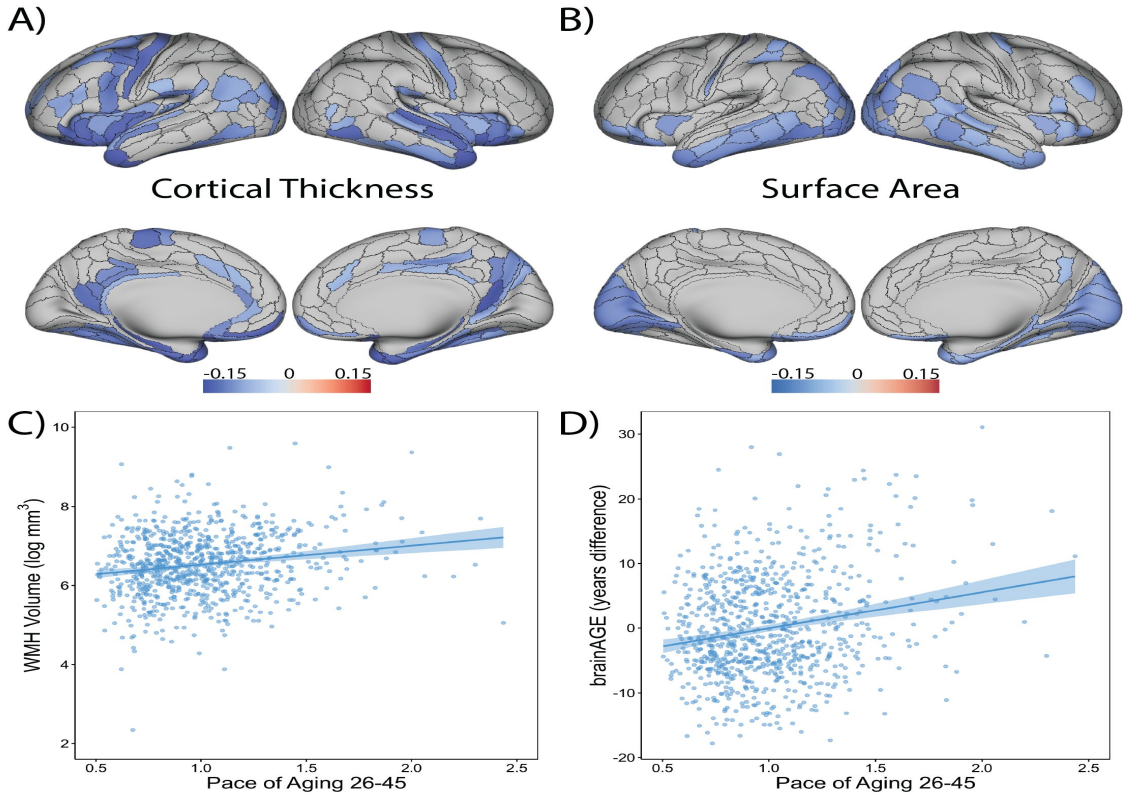


Pace of Aging 26 - 45 $R = 0.16, p < 0.001$

Each plotted point represents 20 study members

TruDiagnostic™

Cortical Thickness and Surface Area of the Brain



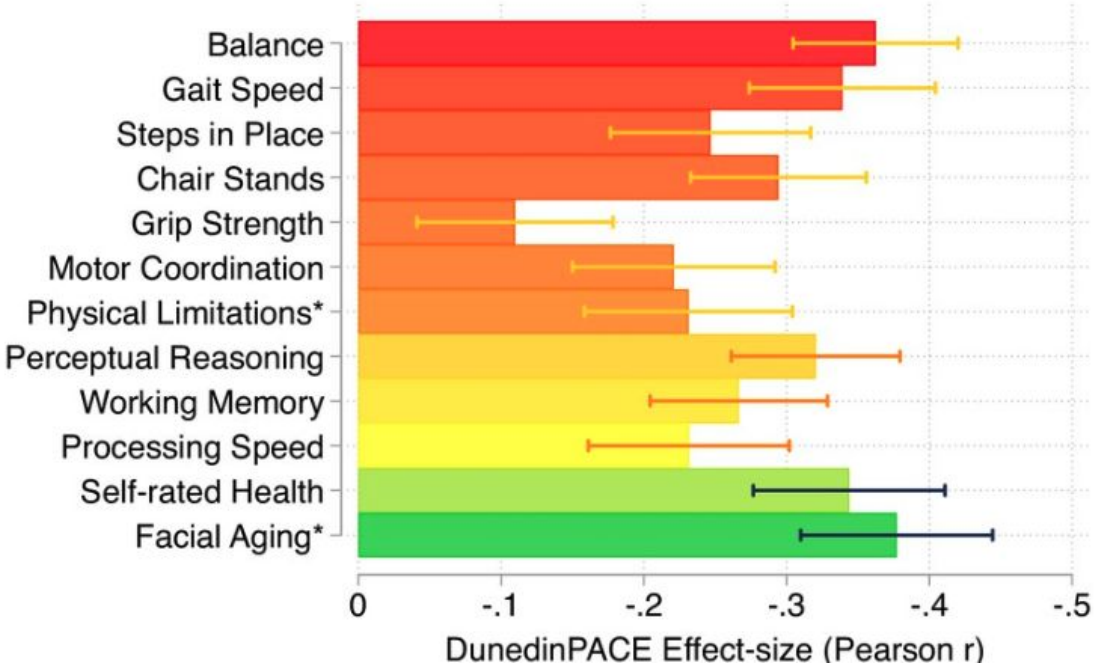
Significant Variation in Facial Aging

10 slowest-aging cohort members

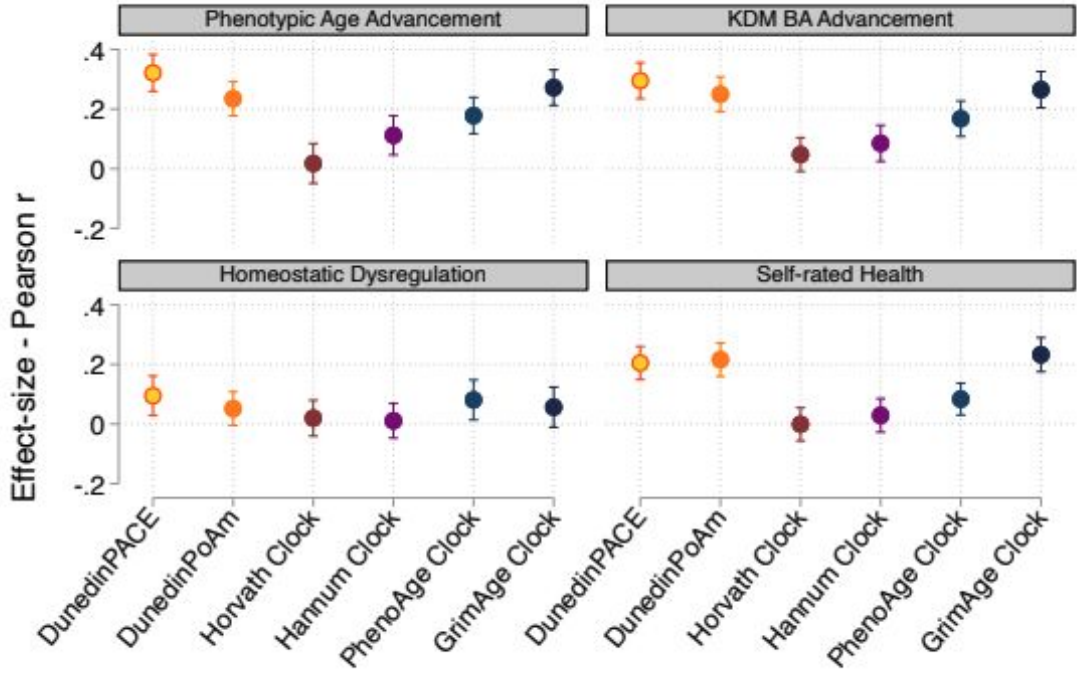
10 average-aging cohort members

10 fastest-aging cohort members

The DunedinPACE has the Highest Correlations to Quality of Life Metrics



The DunedinPACE has the Highest Correlations to Quality of Life Metrics

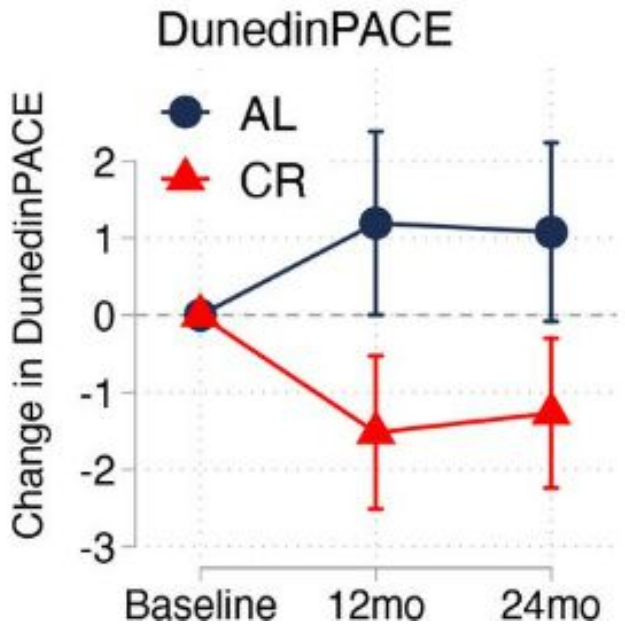


Weakness #1: 1st generation clocks might not respond to validated anti-aging interventions

***This is EXTREMELY important

Biomarker Criteria	Horvath epigenetic age	Hannum epigenetic age	GrimAge	PhenoAge	DunedinPoAm
DNA Methylation Biomarker Calibrated to Detect:	Chronologic Age	Chronologic Age	Biomarkers, Smoking, Death	Phenotypic Age	Pace of Aging (change)
Feasible for use in a clinical trial in older adults?	✓	✓	✓	✓	✓
Robustly associated with chronological age across independent cohorts?	✓	✓	✓	✓	✓
Predict age-related change in function, chronic disease, or death?	✓	✓	✓	✓	✓
Responsive to interventions that beneficially affect the biology of aging?	--	--	--	--	--

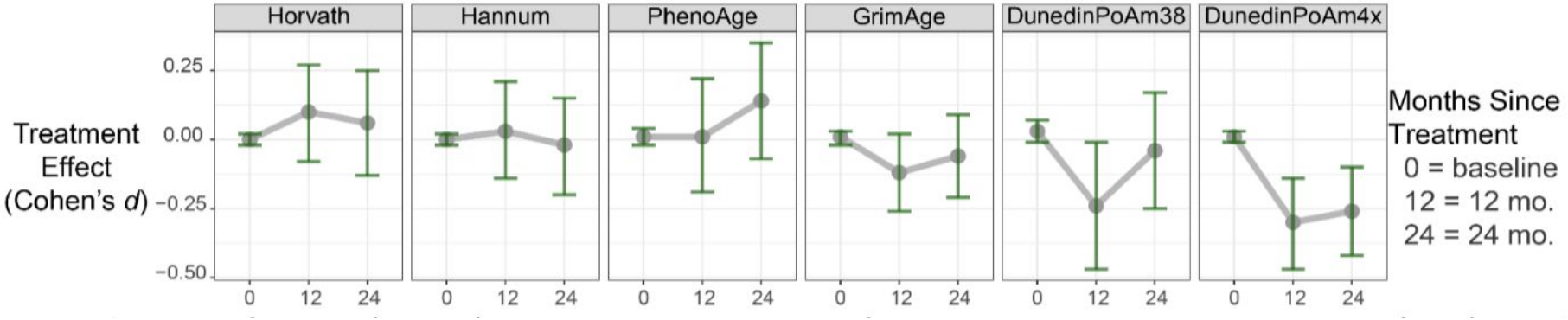
The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan



Change from baseline to 12- and 24-month follow-up in DunedinPACE (Pace of Aging) measures of aging in ad libitum (AL) and caloric restriction (CR) groups in CALERIE Trial (Waziry, R., et al.)

The DunedinPACE is modifiable by interventions which we already KNOW improve healthspan and lifespan

CALERIE RCT of caloric restriction (N=197)



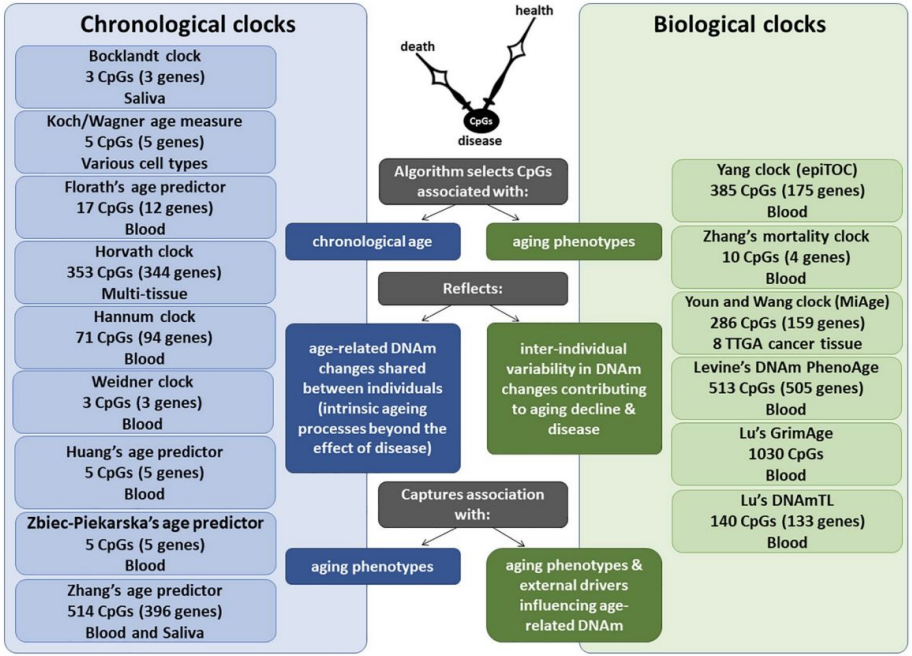
The History of Epigenetic Clocks

Landscape of Clocks

1st Gen. Clock
Trained using **Chronological Age**
Age ~ CpG Methylation + Age + Sex + …

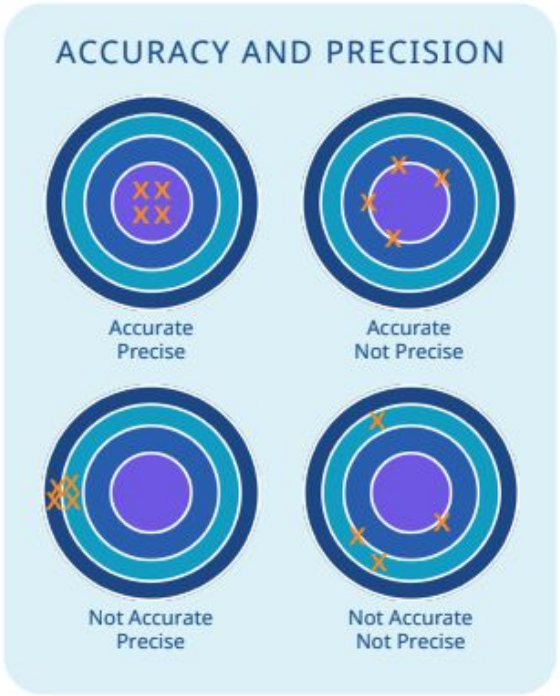
2nd Gen. Clock
Trained using **Aging Phenotypes**
Biomarker ~ CpG Methylation + Age + Sex + …

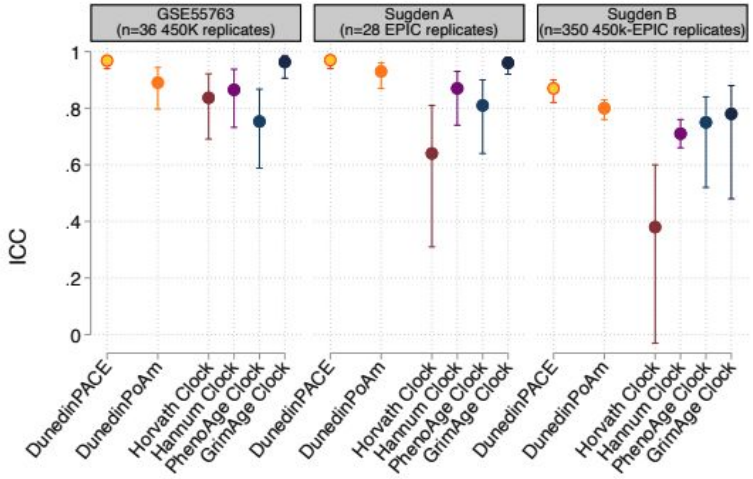
3rd Gen. Clock
Trained using Aging Phenotype and measurements, **produces a instantaneous rate of aging**



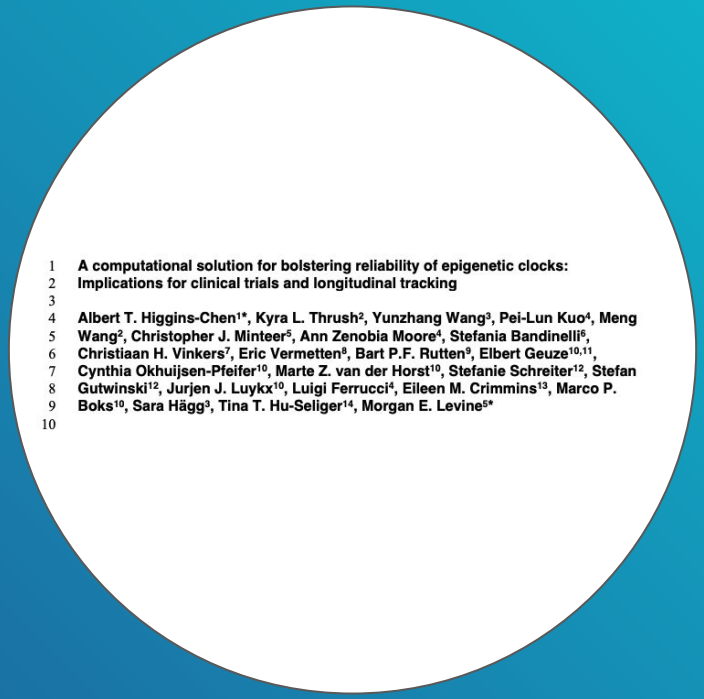
Bergesma and Rogaeva, 2020

The DunedinPACE is the Most Precise Clock





ICC	Interpretation
<0.5	Poor agreement
0.5 to <0.75	Moderate agreement
0.75 to <0.9	Good agreement
0.9 - 1.0	Excellent agreement



A computational solution for bolstering reliability of epigenetic clocks: Implications for clinical trials and longitudinal tracking

Albert T. Higgins-Chen[1*], Kyra L. Thrush[2], Yunzhang Wang[3], Pei-Lun Kuo[4], Meng Wang[2], Christopher J. Minteer[5], Ann Zenobia Moore[4], Stefania Bandinelli[6], Christiaan H. Vinkers[7], Eric Vermetten[8], Bart P.F. Rutten[9], Elbert Geuze[10,11], Cynthia Okhuijsen-Pfeifer[10], Marte Z. van der Horst[10], Stefanie Schreiter[12], Stefan Gutwinski[12], Jurjen J. Luykx[10], Luigi Ferrucci[4], Eileen M. Crimmins[13], Marco P. Boks[10], Sara Hägg[3], Tina T. Hu-Seliger[14], Morgan E. Levine[5*]

Impressive Clock Improvements

We mentioned that one problem of the clocks in noise from each sample and the need for large cohorts to analyze interventions.

New improvements at Yale have increase the precision of the published clocks significantly.

Using **principal component (PC)** analysis, they have been able to increase all ICC values above .95 which is considered excellent.

It is a major step forward and reduces sample sizes needed for statistical analysis by approximately 1/20th.

It needs much more CpG coverage. Around 80,000 CpGs.

Methylation: Strength and Weaknesses

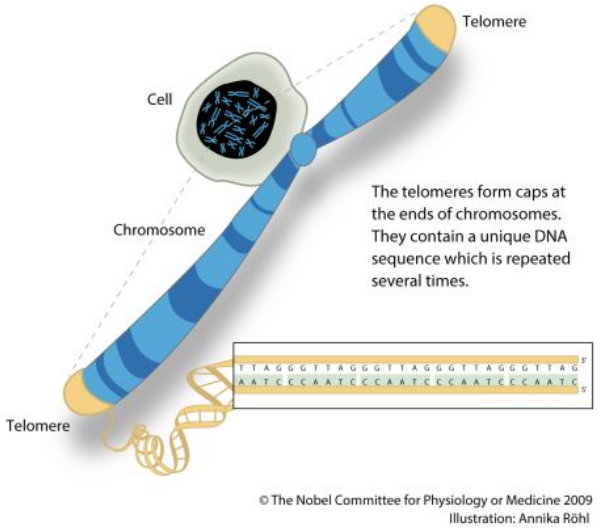
Strengths	Weaknesses
• Highest ICC Values • Highest Hazard Ratios to Disease • Most Validated • Most Interventional Studies • Phenotypically trained • Commercially Validated Algorithms Available • Many Different Reporting Insights	• 1st generation clocks might not respond to validated anti-aging interventions • Immune cells can confound • Causal? • Precision has been traditionally poor • Difference among the many clocks are confusing

Telomere: Strength and Weaknesses

Strengths	Weaknesses
• The Most Validated • Commercially Validated Testing is Available	• Type of cells can confound • Lowest Hazard ratios to disease • Causal? • Correlation to age (r2) is poor • 2 different methods (qPCR vs FISH) provide very different results • Critically short telomeres might be more important

Telomeres: What does this tell us?

- All cells have have a finite replicative potential; it is predictable based on the length of telomere repeat DNA.
- Telomeres define the ends of chromosomes and function to preserve genome integrity; they are comprised of TTAGGG sequences that are bound by specialized proteins.
- Telomere length (TL) shortens during DNA replication and, at a critical threshold, the shortest telomere(s) activate a DNA damage response that signals cell death or a permanent cell cycle arrest, known as cellular senescence
- The observations in cultured cells, and the fact that TL shortens with aging, have led to a hypothesized role for telomere shortening in human aging and age-related disease; **however, the short TL threshold that is clinically relevant for disease risk is not known, and whether TL measurement can influence treatment decisions in clinical settings has not been determined.**



The Most Common Test for Biological Age: Telomeres

- Since the number of cell replication *in vivo* increases with age, telomere length (TL) is negatively correlated with age of proliferating somatic cells. Meta-analysis of 124 cross-sectional studies and 5 longitudinal studies showed that the correlation between leukocyte telomere length (LTL) and age ranges between r=-0.295 and r=-0.338 across adults

- Inherited deficiencies where telomere analysis is used in clinical diagnosis and to guide treatment include bone marrow failure, dyskeratosis congenita, aplastic anemia, acute myeloid leukemia, immune deficiencies, and pulmonary fibrosis.

RESEARCH ARTICLE

Diagnostic utility of telomere length testing in a hospital-based setting

Jonathan K. Alder, Vidya Sagar Hanumanthu, Margaret A. Strong, Amy E. DeZern, Susan E. Stanley, Clifford M. Takemoto, Ludmila Danilova, Carolyn D. Applegate, Stephen G. Bolton, David W. Mohr, Robert A. Brodsky, James F. Casella, Carol W. Greider, J. Brooks Jackson, and Mary Armanios

PNAS March 6, 2018 115 (10) E2358-E2365; first published February 20, 2018 https://doi.org/10.1073/pnas.1720427115

Contributed by Carol W. Greider, January 9, 2018 (sent for review November 28, 2017; reviewed by Thomas R. Cech and Agata Smogorzewska)

Telomere Summary

"Briefly, telomere length is extensively validated but has low predictive power."

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journal homepage: www.ebiomedicine.com

Review

Biological Age Predictors

Juulia Jylhävä, Nancy L. Pedersen, Sara Hägg *

Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

ARTICLE INFO

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Telomere length

ABSTRACT

The search for reliable indicators of biological age, rather than chronological age, has been ongoing for over three decades, and until recently, largely without success. Advances in the fields of molecular biology have increased the variety of potential candidate biomarkers that may be considered as biological age predictors. In this review, we summarize current state-of-the-art findings considering six potential types of biological age predictors: epigenetic clocks, telomere length, transcriptomic predictors, proteomic predictors, metabolomics-based predictors, and composite biomarker predictors. Promising developments consider multiple combinations of these various types of predictors, which may shed light on the aging process and provide further understanding of what contributes to healthy aging. Thus far, the most promising, new biological age predictor is the epigenetic clock; however its true value as a biomarker of aging requires longitudinal confirmation.

Leukocyte Telomere Length:
An Epigenetic Predictor
(accuracy/caveats)

Leukocyte DNAmTL (telomere length) is applicable across the entire age spectrum and is more strongly associated with age than measured leukocyte TL (LTL) **(*r* ~-0.75 for DNAmTL versus *r* ~ -0.35 for LTL).**

DNA methylation-based estimator of telomere length

Ake T. Lu,[1] Anne Seeboth,[2] Pei-Chien Tsai,[3,4,5] Dianjianyi Sun,[6,7] Austin Quach,[1] Alex P. Reiner,[8] Charles Kooperberg,[8] Luigi Ferrucci,[9] Lifang Hou,[10] Andrea A. Baccarelli,[11] Yun Li,[12] Sarah E. Harris,[13,14] Janie Corley,[13,14] Adele Taylor,[13,14] Ian J. Deary,[13,14] James D. Stewart,[15] Eric A. Whitsel,[15,16] Themistocles L. Assimes,[17,18] Wei Chen,[7] Shengxu Li,[19] Massimo Mangino,[3] Jordana T. Bell,[3] James G. Wilson,[20] Abraham Aviv,[21] Riccardo E. Marioni,[2,13] Kenneth Raj,[22,*] and Steve Horvath[1,23,*]

Epigenetic Leukocyte Telomere Length:

A More Accurate Predictor of Health Outcomes

DNAmTL outperforms LTL in predicting

- Time-to-death (p=2.5E-20)
- Time-to-coronary heart disease (p=6.6E-5)
- Time-to-congestive heart failure (p=3.5E-6)
- Association with smoking history (p=1.21E-17)

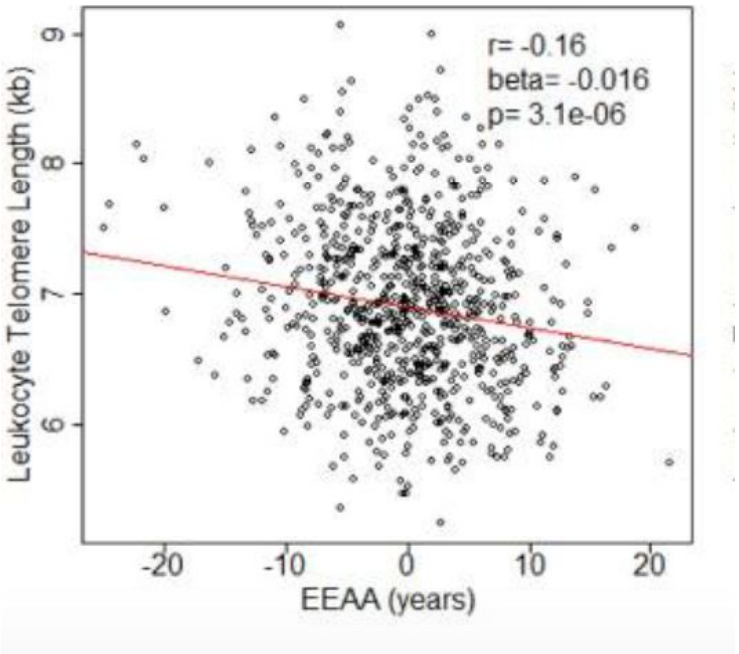
DNAmTL is not only an epigenetic biomarker of replicative history of cells, but a useful marker of age-related pathologies that are associated with it

Leukocyte Telomere Length:

Shorter LTL is associated with increased EEAA (r=-0.16, p=3.1x10-6). LTL is inversely related to proportions of memory CD8+ T cells (p=4.04x10-16) and positively related to proportions of naive CD8+ T cells.

Blood that contains more memory CD8+ T cells and less naive CD8+ T cells would display a relatively shorter LTL and older DNA methylation age.

EEAA is highly predictive of all-cause mortality. Epigenetic mortality risk is strongly associated with telomere length.



Better together!

From the previous slide we know that chronological clocks, phenotypic clocks, and telomere length don't really correlate well with each other meaning they represent different processes.

"Evidence that TL and epigenetic clock estimates are independent predictors of chronological age and mortality risk was obtained in the study by Marioni et al. (2018) performed in two Scottish cohorts aged from 70 to 90 years.

*In both cohorts studied, combined whole-blood TL and DNAm age explained more variance in age than each of them individually. I**n a combined cohort analysis, TL and DNAm age explained 2.8 and 28.5% of the variance in age, respectively, and jointly they explained 29.5%.** Also in a combined cohort, one standard deviation increase in a baseline DNAm age was associated with a 25% increased mortality risk ($p < 0.001$) while in the same model, one standard deviation increase in a baseline TL was independently associated with an 11% reduced mortality risk only ($p = 0.05$)."*

Glycans: Strength and Weaknesses

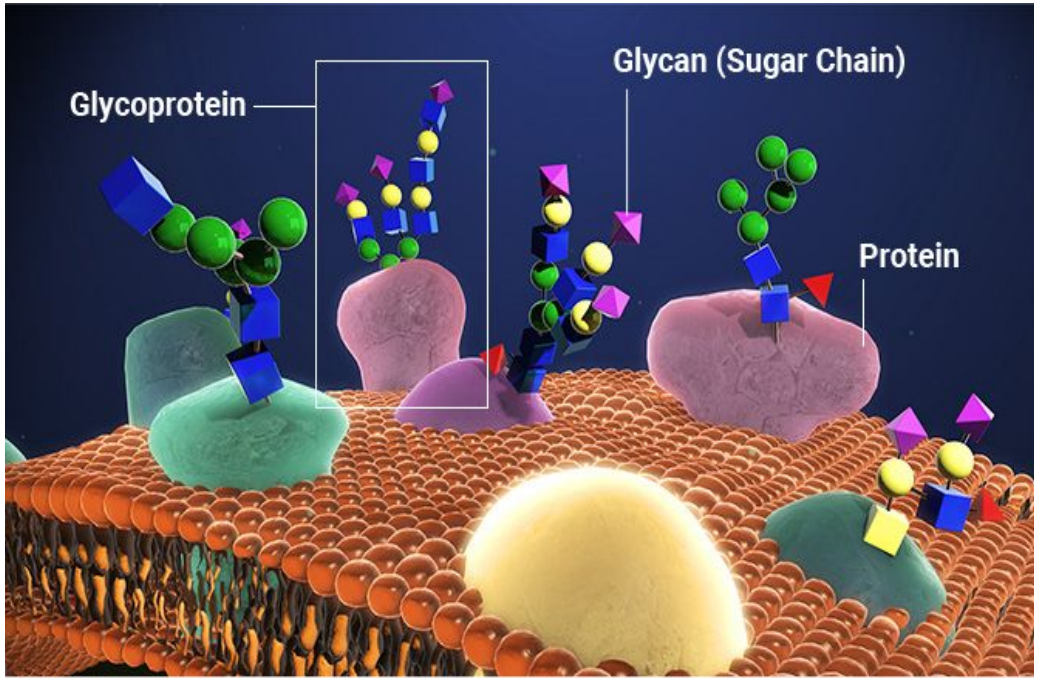
Strengths	Weaknesses
• Some Interventional Studies • Commercially Validated Algorithms Available • Many Different Reporting Insights • Highly connected to many diseases	• Too modifiable by intervention • No large scale validation studies • No ICC values for individual markers >.80 • Lowest powered training dataset of any omic clocks (n= 2217) • Lower r2 than many other omics (Max .80) • Not phenotypically trained • Larger heritability estimates than methylation (39% versus 20% for newer clocks, 71% with age)

What are Glycans?

Glycans, also called polysaccharides, are carbohydrate-based polymers made by all living organisms.

They are sugar-based polymers that coat cells and decorate most proteins forming glycoproteins.

Glycans are essential biomolecules serving structure, energy storage and system regulatory purposes.



R2 is relatively low

J Gerontol A Biol Sci Med Sci. 2014 Jul; 69(7): 779–789.
Published online 2013 Dec 10. doi: 10.1093/gerona/glt190
PMCID: PMC4049143
PMID: 24325898
Editor's choice

Glycans Are a Novel Biomarker of Chronological and Biological Ages

Jasminka Krištić,[1,*] Frano Vučković,[1,*] Cristina Menni,[2] Lucija Klarić,[1] Toma Keser,[3] Ivona Beceheli,[1] Maja Pučić-Baković,[1] Mislav Novokmet,[1] Massimo Mangino,[2] Kujtim Thaqi,[1] Pavao Rudan,[4] Natalija Novokmet,[4] Jelena Šarac,[4] Saša Missoni,[4] Ivana Kolčić,[5] Ozren Polašek,[5] Igor Rudan,[6] Harry Campbell,[6] Caroline Hayward,[7] Yurii Aulchenko,[8] Ana Valdes,[2] James F. Wilson,[6] Olga Gornik,[3] Dragan Primorac,[9] Vlatka Zoldoš,[10] Tim Spector,[2] and Gordan Lauc[1,3]

This study on the glycan age index showed the max R2 to chronological age was .80. It also had the lowest training size of an biomarker at n= 2217.

This could mean its R2 could get much better in larger cohorts.

Goodness-of-Fit and Spearman's Correlations of Chronological Age and Age Predicted by Various Models

		Population		Female		Male	
Training	**Test**	R^2	**Correlation**	R^2	**Correlation**	R^2	**Correlation**
GlycanAge Index							
Orkney	Orkney	57.8% [54.1–61.3%]	.76 [.73 to .78]	64.0% [60.0–68.1%]	.80 [.77 to 0.82]	49.4% [41.9–56.7%]	.70 [.64 to .75]
Orkney	Korcula	41.3%	.64	50.6%	.71	25.2%	.50
Orkney	Vis	41.5%	.64	49.1%	.70	31.4%	.56
Orkney	TwinsUK	48.0%	.69	48.0%	.69	NA	NA
Korcula	Korcula	42.9% [34.2–49.7%]	.65 [.58 to .70]	51.1% [40.5–58.9%]	.71 [.63 to 0.76]	26.8% [16.1–36.3%]	.51 [.40 to .60]
Vis	Vis	43.0% [36.6–51.0%]	.65 [.60 to .71]	49.8% [41.3–57.8%]	.70 [.64 to .76]	34.6% [24.6–46.2%]	.58 [.49 to .67]
TwinsUK	TwinsUK	49.5% [42.9–55.3%]	.70 [.65 to 0.74]	49.5% [42.9–55.3%]	.70 [.65 to .74]	NA	NA
Combined Glycan Age Index							
Orkney	Orkney	71.3% [68.2–73.9%]	.84 [.83 to 0.86]	75.7% [72.7–78.4%]	.87 [.85 to .89]	63.7% [57.1–69.2%]	.80 [.76 to .83]
Orkney	Korcula	64.5%	.80	69.5%	.83	56.3%	.75
Korcula	Korcula	65.2% [59.7–70.0%]	.81 [.77 to 0.84]	69.6% [63.8–74.8%]	.83 [.80 to .86]	57.4% [46.5–67.0%]	.76 [.68 to .82]

ICCs could be problematic

Table 2

Heritability estimates and 95% confidence intervals for IgG glycan traits adjusted for age and batch.

	MZ		DZ			
Glycan Trait	**Mean(SD)***	**ICC[95%CI]**	**Mean(SD)***	**ICC[95%CI]**	**Best model**	**A[95%CI**
GP1	0.1(0.06)	0.54[0.44,0.63]	0.11(0.06)	0.38[0.28,0.47]	AE	0.58[0.49,
GP2	0.48(0.27)	0.68[0.61,0.75]	0.53(0.34)	0.24[0.13,0.34]	AE	0.72[0.64,
GP4	19.41(6.45)	0.73[0.66,0.79]	19.26(5.63)	0.39[0.29,0.49]	AE	0.70[0.64,
GP5	0.32(0.15)	0.63[0.54,0.71]	0.4(0.17)	0.41[0.32,0.50]	AE	0.72[0.65,
GP6	5.33(1.77)	0.76[0.70,0.82]	5.32(1.68)	0.33[0.23,0.43]	AE	0.75[0.69,
GP7	0.54(0.24)	0.66[0.58,0.73]	0.62(0.31)	0.33[0.23,0.43]	AE	0.73[0.67,
GP8	18.97(1.74)	0.73[0.67,0.80]	19.19(1.83)	0.30[0.20,0.41]	AE	0.74[0.68,
GP9	9.98(1.42)	0.74[0.68,0.80]	9.99(1.44)	0.41[0.32,0.51]	AE	0.75[0.69,
GP10	6(1.21)	0.76[0.71,0.82]	5.99(1.19)	0.37[0.28,0.47]	AE	0.76[0.70,
GP11	0.84(0.22)	0.73[0.67,0.79]	0.86(0.2)	0.38[0.28,0.48]	AE	0.71[0.65,
GP12	0.67(0.32)	0.54[0.44,0.63]	0.71(0.38)	0.32[0.22,0.43]	AE	0.60[0.51,
GP13	0.5(0.22)	0.64[0.57,0.72]	0.62(0.23)	0.46[0.37,0.55]	AE	0.70[0.63,

PLoS One. 2013; 8(12): e82558.
Published online 2013 Dec 6. doi: 10.1371/journal.pone.0082558
PMCID: PMC3855797
PMID: 24324808

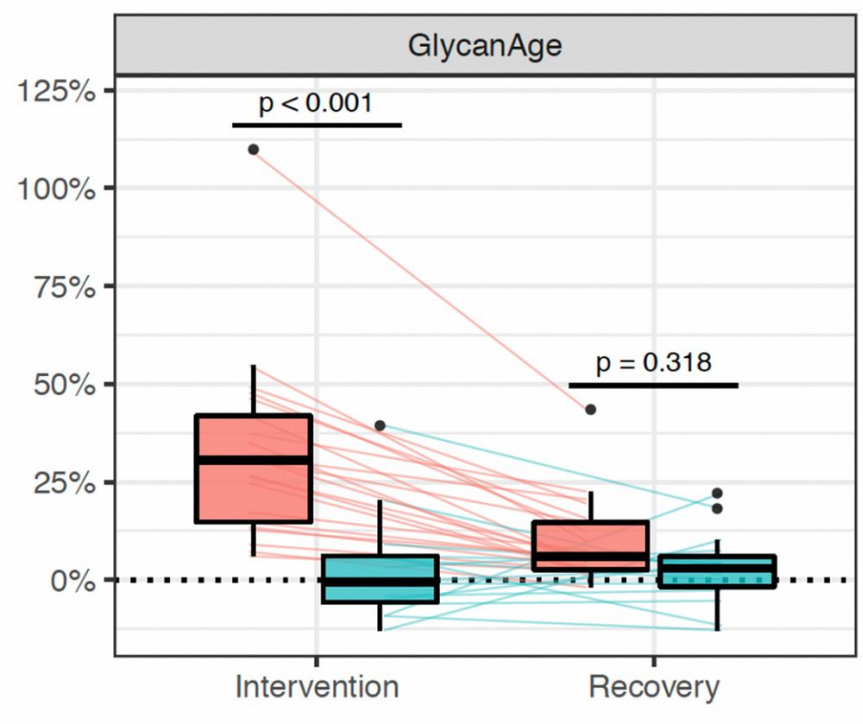
Glycosylation of Immunoglobulin G: Role of Genetic and Epigenetic Influences

Cristina Menni,[#1,*] Toma Keser,[#2] Massimo Mangino,[1] Jordana T. Bell,[1] Idil Erte,[1] Irena Akmačić,[3] Frano Vučković,[3] Maja Pučić Baković,[3] Olga Gornik,[2] Mark I. McCarthy,[4,5] Vlatka Zoldoš,[6,¶] Tim D. Spector,[1,¶] Gordan Lauc,[2,3,¶] and Ana M. Valdes[1,7,¶]

It was hard to find any reproducibility data on GlycanAge and ICCs, however, the study above listed ICCs for most common glycan and none had an ICC above .80.

Considering GlycanAge uses many of these, it might compound. However, this has not been published or released and is speculation.

Estrogen effects on GlycanAge



Research Paper | Volume 12, Issue 19 | pp 19756—19765

Effects of estradiol on biological age measured using the glycan age index

Julija Jurić[1] , Wendy M. Kohrt[2,3] , Domagoj Kifer[4] , Kathleen M Gavin[2,3] , Marija Pezer[1] , Peter A. Nigrovic[5,6] , Gordan Lauc[1,4]

This study looked at estrogen deprivation and subsequent replacement with estrogen. Thus, it is not exactly similar to estrogen replacement due to menopause or age. However, they were able to show that those with estrogen supplementation were not aged while those still with low estrogen levels aged approximately 9.5 years.

In cohorts we have dual testing for, we often see major decreases in Glycan age with estrogen therapy often up to 20 years.

No Large Scale Validation Studies to Disease

Key Publications

- Glycans Novel Biomarkers →
- Glycans & Life Expectancy →
- Glycans & Loss of Kidney Function →
- Weight loss can reduce immune age →
- **Effects of Estrogen on GlycanAge** →
- Glycans & Interval training →
- Competitive bodybuilding & immunosuppression →
- Glycans & High Blood Pressure →
- All Publications →

We could find no large scale study of the GlycanAge index to disease in large validation cohorts.

Glycans are certainly an amazing biomarker and have shown associations to many other diseases. However, the GycanAge index is not as we validated.

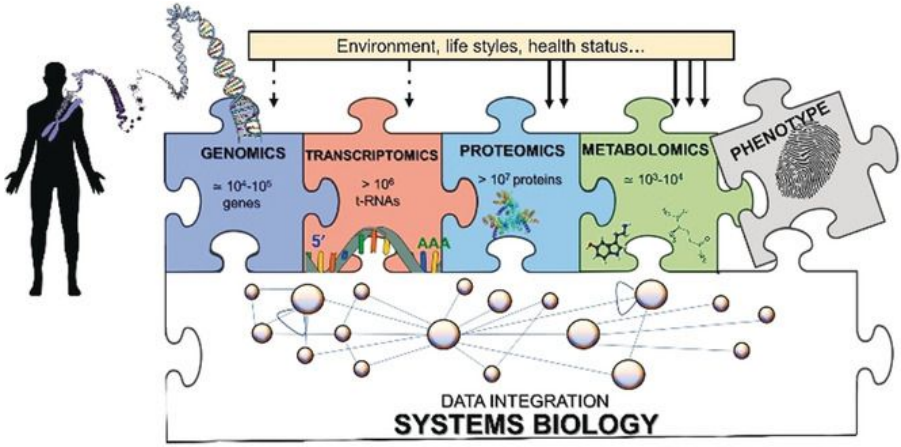
Metabolomics: Strength and Weaknesses

Strengths	Weaknesses
• High Hazard Ratios to Disease • Validated Algorithms Available (not commercial) • Many Different Reporting Insights	• No clocks commercially available • • Very few clocks with multiple interventional studies • • No clocks with interventional data or precision data

Metabolomic Clocks

What is the metabolome?

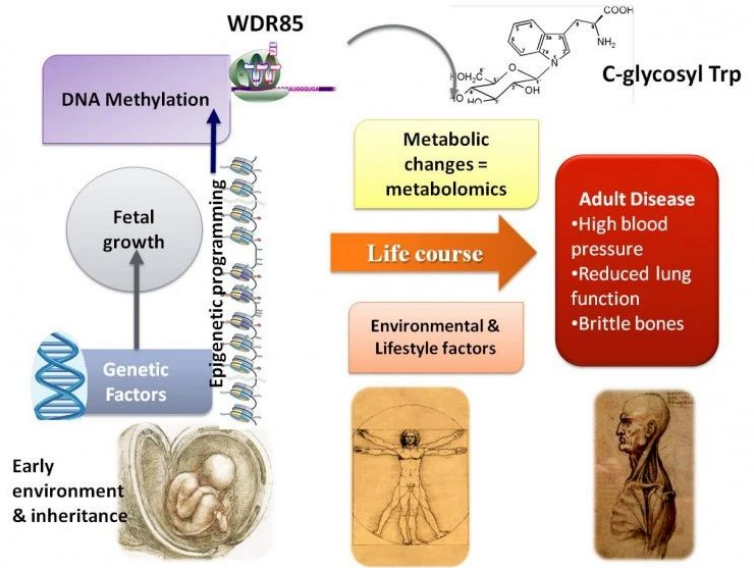
- Metabolomics is the scientific study of chemical processes involving metabolites, the small molecule substrates, intermediates and products of metabolism. Specifically, metabolomics is the "systematic study of the unique chemical fingerprints that specific cellular processes leave behind", the study of their small-molecule metabolite profiles.
- Metabolic profiling can give an instantaneous snapshot of the physiology of that cell, and thus, metabolomics provides a direct "functional readout of the physiological state" of an organism.
- Relatively few studies have analyzed associations with age on the metabolome and they were conducted using different measurement techniques. Yu and colleagues used a targeted mass-spectrometry method identifying 131 metabolites in fasting serum, where 11 were independently associated with age in females after BMI adjustments (Yu et al., 2012).
- Later, the same groups combined analyses of non-targeted mass-spectrometry and age using the Metabolon platform (Menni et al., 2013).



Metabolomic Clocks

- In that study, 22 independent age-associated metabolites, mostly lipids and amino acids, were found. One selected metabolite, C-glyTrp, was associated with age-related traits such as lung function and hip bone mineral density after adjustments for age. In a study from 2016 by Hertel and colleagues, a proton nuclear magnetic resonance (H1 NMR) spectroscopy investigation in human urine samples quantified 59 metabolites (Hertel et al., 2016).

- Construction of a Metabolic Age Score included all metabolites as predictors and age as the outcome. The metabolic age score was validated and replicated in two independent cohorts, and found to associate with clinical outcomes independent of age, e.g., kidney malfunction, high HbA1c levels, and hyperglyceridemia. Importantly, survival analysis showed that individuals in the first tertile of the score (lower biological age) had higher all-cause survival rates, and that the prediction added value over commonly known risk factors.



Proteomics: Strength and Weaknesses

Strengths	Weaknesses
• Well Powered Studies • Validated Algorithms Available • Many Different Reporting Insights	• DIA versus DDA Approach • Targeted Versus untargeted • Precision has been traditionally poor • No clock which has been highly validated in large cohorts

Proteomics Clocks

"There were cases of substantial divergence between participants' chronological and physiological age — for example, among the subjects in the LonGenity study, with their genetic proclivity toward exceptionally good health in what for most of us is advanced old age.

"We had data on hand-grip strength and cognitive function for that group of people," Wyss-Coray. "Those with stronger hand grips and better measured cognition were estimated by our plasma-protein clock to be younger than they actually were."

<u>However, the protein-derived age variable itself was not tested for associations with health outcomes.</u>

Stanford scientists reliably predict people's age by measuring proteins in blood

Protein levels in people's blood can predict their age, a Stanford study has found. The study also found that aging isn't a smoothly continuous process.

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The carnival worker who tries to guess your age relies on aspects of your appearance, such as your posture and whether any wrinkles emanate from the corners of your eyes and lips. If the carny's guess is more than a few years off, you win a stuffed koala.

But a team of Stanford University School of Medicine scientists doesn't need to know how you look to guess your age. Instead, it watches a kind of physiological clock: the levels of 373 proteins circulating in your blood. If the clock is off, you don't win a plush toy. But you may find out important things about your health.

Tony Wyss-Coray is the senior author of a study that found protein levels in people's blood can predict their age.
Norbert von der Groeben

"We've known for a long time that measuring certain proteins in the blood can give you information about a person's health status — lipoproteins for cardiovascular health, for example," said Tony Wyss-Coray, PhD, professor of neurology and neurological sciences, the D. H. Chen Professor II and co-director of the Stanford Alzheimer's Disease Research Center. "But it hasn't been appreciated that so many different proteins' levels — roughly a third of all the ones we looked at — change markedly with advancing age."

Competitive Landscape | Leaders in Biological Age Testing

Platform Technology	Validation Studies	Commercially Available Published Algorithms	R2 to Age	Hazard Ratio	Response to Validated (non-disease) Aging Intervention	ICC Values	Commercial Phenotypically Trained?	Summary
Methylation	<100s for each clock	✓	>.999 in new clocks	Highest hazard ratios to all age related disease, best for mortality prediction	Yes, caloric restriction in CALERIE study with >2,000 Healthy patients	>.99 in Most Clocks	✓	Some great low cost methods, not many commercially available, best methods require multiple data points, physical function measures, questionnaires.
Proteomics	<5 for each clock	✗	Best = .98 (not available)	High hazard ratios to Stroke and Cancer	??	.79	✗	Generally a promising scalable biomarker but currently very expensive Still less validated and less predictive than methylation clocks. No commercial option.
Metabolomics	<5 for each clock	✗	.86	Not well validated	??	.85-.93	✗	Generally a promising scalable biomarker but expensive at the moment to get the data for all the clocks. Still less validated and less predictive than methylation clocks.
Telomeres	>1,000	✗	0.295 - 0.338	Worst aging hazard ratios	Adjusting for covariates & cytomegalovirus, we observed shorter blood mononuclear cell telomeres in the CR group (p=0.012) .	.60-80	✗	Extensively well validated but not highly predictive.
Glycans (proteomic)	30+	✓	.645-.713	No large scale meta analysis to disease outcomes	There has be a single interventional study, however, not many separated from disease	??	✗	Glycans look to be an amazing biomarker but Glycan age has few validation studies to disease. Might be too modifiable with therapy.
Blood based Analysis	<20 for each clock	✗	.97 is best (ENABL) Most lower	Variable depending on method. Some great methods but require expansive biomarkers.	??	<.80 due to many lab methods	✗	Some great low cost methods, not many commercially available, best methods require multiple data points, physical function measures, questionnaires.

Methylation Aging Test Landscape | Quick Comparison

Company	# of Age Outputs	Published Algorithms	Data Testing Size	Collection Method (Blood is only validated method)	Response to Validated (non-disease) Aging Intervention	Immune Deconvolution?	ICC Values	Hazard Ratios to Disease?	Generation Clock?
TruDiagnostic™ The Epigenetic Company	8	✓	Approximately 850k CpGs	Blood	✓	✓	All >.98	Best of any published methylation clock	2nd and 3rd
myDNAge®	1	✗	2,000 CpGs	Blood Saliva	✗	✗	Not Published	Not Published	1st
ELYSIUM	1	✗	Approximately 350k CpGs	Saliva	✗	✗	Not Published	Not Published	1st
DONOTAGE	1	✗	??	Saliva	✗	✗	Not Published	Not Published	1st
epiAge	1	✗	300 CpGs	Saliva	✗	✗	Not Published	Not Published	1st
Tally Health	1	✗	5,000 CpGs	Saliva	✗	✗	Not Published	Not Published	1st

Upcoming TruAge Improvements

1. We will remove 1st generation clocks entirely.
2. We will report aging of different organs directly.
3. We will have a senescence burden predictor.
4. All of our algorithms will be controlled with most advanced immune cell subsets. The Buck institute will also release an immune controlled algorithm soon!
5. We hope to offer causal and protective aging algorithms for causal information.
6. We will publish novel metabolomic and proteomic clocks and have methylation predictors of these. This will culminate in a comprehensive Multi-Omic aging clock trains for time until death and phenotypic aging.

Questions?

Feel free to contact me
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